13544

044081

Chemical:

PC Code:
HED File Code
Memo Date: 05/06/87
File ID: 00000000
Accession Number: 000-00-0051

HED Records Reference Center
05/30/2002

CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

MAY 6 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review of a mutagenicity study with Benodanil Technical
EPA ID #372-AU Caswell No. 501 AB
EPA Accession No. 264301 Tox Proj No. 2342

TO: Ms. Lois Rossi, PM #21
Registration Division (TS-767c)

FROM: Quang Q. Bui, Ph.D.
Acting Head, Review Section V
Toxicology Branch/HED (TS-769c) Quang Bui 4/28/87

THRU: Irving Mauer, Ph.D.
Geneticist
Toxicology Branch/HED (TS-769c) 4-28-87
and
Theodore M. Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769c)

Registrant: Mallinckrodt Inc.,
St. Louis, Missouri 63147

Action Requested: Review an in-vitro cytogenetics study with Technical Benodanil in Chinese Hamster Ovary cells (Hill Top Res. # 85-1017-15, dated 7/14/1986)

RECOMMEDATION

Under the conditions of this assay, Technical Benodanil (97.5% a.i) was clastogenic in the in vitro cytogenetic assay using the Chinese Hamster Ovary Cells in both absence and presence of metabolic activation.

A statistically significant increase in the total aberration frequencies and total abnormal cells was found at the 100 ug/ml dosage level. The investigators claimed that "the presence of a few heavily damaged cells at 33 ug/ml and 11 ug/ml suggests that it is also clastogenic at these concentrations".

It is recommended that this study be classified as Acceptable Data.

DATA EVALUATION REPORT

Chemical: Benodanil
Test Material: Technical 97.5% pure
(grey, granular/powdery substance)

Study Identification:

"In Vitro Cytogenetics Study: Chromosomal Aberrations in Chinese Hamster Ovary (CHO) Cells"

Testing Facility: Hill Top Research Inc.,
Final Report No.: 85-1017-15
Report Date: 7/14/86
Study Authors: G.C. Lavelle et al.

EPA Accession No.: 264301

Reviewed by: Quang Q. Bui, PhD., DABT.
Acting Head, Review Section V
Toxicology Branch/HED

RECOMMENDATION AND CONCLUSION

The authors indicated that this study was conducted three times. The first and second trials yielded chromosomal slides not suitable for scoring due to the lack of an adequate number of metaphase cells. Only the findings of the third trial were presented in this final report.

A statistically significant increase ($p < 0.005$) in the total aberration frequencies and total abnormal cells (including achromatic lesions) was noted at the 100 ug/ml in both presence and absence of metabolic activation. The investigators indicated that "the presence of a few heavily damaged cells at 33 and 11 ug/ml suggests that it is also clastogenic at these concentrations".

Under the conditions of this assay, Technical Benodanil was clastogenic in Chinese Hamster Ovary Cells in both presence and absence of metabolic activation.

It is recommended that this study be classified as Acceptable Data.

PROCEDURES

The potential of Technical Benodanil to induce chromosomal aberrations in mammalian cells was investigated in an in vitro cytogenetics test using the Chinese Hamster Ovary (CHO) cells. The test material was assayed at 11, 33, 100, 300, or 600 ug/ml both in the presence and absence of metabolic activation.

1. Cells used: Kl-BH4 (obtained from Dr. A. Li, St. Louis, MO.)
2. Medium used: Ham's F12 supplemented with fetal bovine serum, glutamine, and antibiotics.
3. Negative controls: solvent (DMSO) or S-9
4. Positive controls: Ethylmethanesulfonate (EMS), without S-9
   Cyclophosphamide (CP), with S-9
5. S-9 system: The metabolic activation system was prepared from Aroclor-treated Sprague Dawley rats as described by Ames et al. (1975). The composition of the S-9 mix and the purification methods were described.

RESULTS

1. Toxicity Tests

Test Material ug/ml		Percent Survival ° With S-9	Without S-9
DMSO	1%		100.0
EMS	0.5 ug/ml		24.7
S-9	20 ul/ml	100.0	
CP	25.0	14.0	
Benodanil	11	95.8	87.6
Benodanil	33	84.2	66.9
Benodanil	100	69.8	74.5
Benodanil	300	23.9	18.7

(°) % survival is based on the number of colonies from duplicate cultures, 2 dishes per culture.

2. Dose Selection

The doses of Technical Benodanil used in this assay were 11, 33, 100, 300, and 600 ug/ml. From the data submitted, the dose levels selected apparently were of the correct magnitude for this assay as evidenced by the results from the toxicity tests and by the precipitation at 600 ug/ml.

2. Cloning Efficiency

Test Material	Dose ug/ml	Mean No. Colonies/plate WO S-9	With S-9	% control † WO S-9	With S-9
DMSO	1%	113[139]°	-	100	-
S-9	20	-	111[178]	-	100
EMS	0.5	35 [28]	-	31 [20]	-
CP	25	-	16[25]	-	14[14]
Benodanil	11	115[106]	138[139]	102 [76]	124[78]
Benodanil	33	68[100]	136[107]	60 [72]	123[60]
Benodanil	100	101[87]	122[80]	89 [63]	110[45]
Benodanil	300	12[35]	49[20]	11 [25]	44[11]
Benodanil	600 [a]				

(†) % control = Mean No. colonies x 100/Mean No. colonies for solvent or S-9 control
(°) duplicate plates were used for each of the two cultures; [second culture values]
(a) insoluble and toxic, no data.

From the data on cloning efficiency, a dose-dependent response was noted with clonal toxicity observed at the 300 ug/ml in both presence and absence of metabolic activation. The authors indicated that the test material was insoluble and toxic at the 600 ug/ml dosage level.

3. Cytogenetic Findings

Results of the cytogenetic findings are photocopied from Table 2 of the final report and are attached with this DER.

All types of chromosomal aberrations (chromatid deletions, chromatid interchanges, chromosome deletions, and chromosome exchanges) were recorded, together with achromatic lesions. A higher frequency of chromosomal aberrations was found with the positive controls, EMS (without S-9) and CP (with S-9). A compound-related increase in the total aberration frequencies and total abnormal cells (including achromatic lesions) was noted in the Benodanil plates with a statistical significance noted at the 100 ug/ml dosage level in both presence and absence of metabolic activation.

The data suggest that Technical Benodanil was positive for chromosomal aberrations in the presence and absence of S-9.

TABLE 2

Summary of Cytogenetic Findings for Benodanil Technical in the CHO In Vitro Cytogenetics Assay with and without S-9 Metabolic Activation.[a]

Treatment		No. of Cells	Normal	Chromatid Deletions	Chromatid Inter-Changes	Chromo-some Deletions	Chromo-some Exchanges	Achro-matic Lesions
WITH S-9								
Test Compound	A[b]	120	101	11	4	4	8	4
100 ug/ml	B	90	86	3	0	0	1	1
Test Compound	A	100	93	8	2	0	5	0
33 ug/ml	B	125	117	3	1	0	1	3
Test Compound	A	150	143	6	1	0	2	0
11 ug/ml	B	67	66	0	1	0	0	0
S-9 20 ul/ml	A	150	138	7	2	0	3	1
	B	131	120	2	4	0	4	3
Cyc[c] 25 ug/ml	A	100	94	7	0	0	0	0
	B	136	107	27	2	1	0	8
WITHOUT S-9								
Test Compound	A	150	135	31	1	1	1	3
100 ug/ml	B	73	74	1	1	0	0	1
Test Compound	A	145	136	6	5	0	1	1
33 ug/ml	B	150	140	8	0	5	2	1
Test Compound	A	150	137	6	0	4	4	2
11 ug/ml	B	150	144	5	2	0	0	0
DMSO[c] 1%	A	150	144	1	2	0	1	5
	B	150	140	9	1	4	1	1
EMS[c] 0.5 ul/ml	A	112	100	9	1	0	2	3
	B	145	127	11	3	2	2	5

[a] See the Appendix for detailed tabulation of all cytogenetic findings.

[b] A and B represent duplicate cultures for each test and control group.

[c] Cyc = Cyclophosphamide; DMSO = dimethylsulfoxide; EMS = ethyl methanesulfonate